AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method of expressing an immunotoxin in *Pichia pastoris* that expresses the immunotoxin, the method comprising:

a) growing the a *Pichia pastoris* that expresses the immunotoxin under control of an AOX1 promoter in a growth medium comprising an enzymatic digest of protein and yeast extract; and

b) performing methanol induction on the *Pichia pastoris,* wherein the methanol induction is performed at a temperature of 17.5°C ~~16.5°C~~ and below.

2. (Previously Presented) The method of claim 1, wherein the methanol induction comprises a limited methanol feed of between 0.5-0.75 ml/min (per 10 L initial medium).

3. (Previously Presented) The method of claim 1, wherein the methanol induction comprises a methanol and glycerol containing feed.

4. (Original) The method of claim 3, wherein the ratio of methanol to glycerol in the methanol and glycerol containing feed is about 4:1.

5. (Original) The method of claim 1, wherein the immunotoxin is a fusion protein.

6. (Original) The method of claim 1, wherein the immunotoxin comprises a diphtheria toxin moiety.

7. (Original) The method of claim 6, wherein the diphtheria toxin moiety is truncated.

8. (Original) The method of claim 7, further comprising a CD3 antibody moiety.

9. (Original) The method of claim 8, wherein the immunotoxin comprises A-dmDT390- bisFv(G_4S).

10. (Previously presented) The method of claim 1, wherein the *Pichia pastoris* comprises a mutation in the amino acid sequence of the diphthamide region of EF-2, wherein the mutation prevents ADP ribosylation of EF-2.

11. (Original) The method of claim 1, wherein the enzymatic digest of protein is an enzymatic digest of soy protein.

12. (Currently amended) The method of claim 1, further comprising contacting the *Pichia pastoris* with phenylmethanesulfonyl fluoride and a source of amino acids.

13. (Currently amended) The method of claim 12, wherein the *Pichia pastoris* is contacted with the phenylmethanesulfonyl fluoride and the source of amino acids for at least 2 hours during the methanol induction.

14. (Currently amended) The method of claim 12, wherein the phenylmethanesulfonyl fluoride is dissolved in a 4:1 methanol glycerol induction feed and the concentration of phenylmethanesulfonyl fluoride does not exceed 10 mM.

15. (Original) The method of claim 12, wherein the source of amino acids is a yeast extract.

16. (Currently amended) The method of claim 1, wherein the temperature can be selected from the group of temperatures consisting of 17.5, 17.0, 16.5, 16.0, 15.5, 15.0, 14.5, 14.0, 13.5, 13.0, 12.5, and 12.0°C.

17. (Original) The method of claim 1, wherein the temperature is about 15°C.

18. (Original) The method of claim 1, wherein the composition of the growth medium is about 4% glycerol, about 2% yeast extract, about 2% enzymatic digest of

soy protein, about 1.34% yeast nitrogen base with ammonium sulfate and without amino acids, and about 0.43% PTM1 solution.

19. (Original) The method of claim 18, wherein the growth medium further comprises an antifoaming agent.

20. (Original) The method of claim 19, wherein the antifoaming agent is at a concentration of about 0.01% or greater.

21. (Original) The method of claim 20, wherein the composition of the growth medium is about 4% glycerol, about 2% yeast extract, about 2% enzymatic digest of soy protein, about 1.34% yeast nitrogen base with ammonium sulfate and without amino acids, about 0.43% PTM1 solution and about 0.02% antifoaming agent.

22. (Original) The method of claim 1, wherein dissolved oxygen concentration in the growth medium is maintained at a value of 40% or higher.

23. (Original) The method of claim 1, wherein the growth step is at a pH of about 3.5 and the methanol induction step is at a pH of about 7.0.

24. (Original) The method of claim 1, wherein the methanol induction step is performed for between about 22 and 288 h.

25. (Currently amended) A method of expressing an immunotoxin in *Pichia pastoris* that expresses the immunotoxin, the method comprising:

a) growing a the *Pichia pastoris* that expresses an immunotoxin under control of an AOX1 promoter in a growth medium comprising an enzymatic digest of protein and yeast extract;

b) performing methanol induction on ~~of~~ the *Pichia pastoris*, wherein the methanol induction comprises a limited methanol feed of 0.5-0.75 ml/min/10L of initial volume of the growth medium, wherein the induction is performed at a

temperature 17.5°C ~~16.5~~°C and below, wherein an antifoaming agent supplied in the growth medium at a concentration of up to 0.07%, wherein agitation is maintained at about 400 RPM during the induction step, and wherein the induction step is performed for between about 22 and 288 h.

26. (Currently amended) A method of expressing an immunotoxin in *Pichia pastoris* that expresses the immunotoxin, the method comprising:

a) growing the ~~a~~ *Pichia pastoris* that expresses an immunotoxin under control of an AOX1 promoter in a growth medium comprising about 4% glycerol, about 2% yeast extract, about 2% enzymatic digest of soy protein, about 1.34% yeast nitrogen base with ammonium sulfate and without amino acids, and about 0.43% PTM1 solution, wherein the growth occurs at a pH of about 3.5, and wherein the dissolved oxygen concentration in the growth medium is maintained at a value of 40% or higher; and

b) performing methanol induction on ~~of~~ the *Pichia pastoris*, wherein the methanol induction comprises a limited methanol feed of 0.5-0.75 ml/min/10L of initial volume of growth medium, wherein the induction is performed at a temperature is 15°C, wherein the pH of the growth medium during the induction step is about 7.0, wherein antifoaming agent supplied in the growth medium at a concentration of 0.02%, wherein the agitation is maintained at about 400 RPM during the induction step, and wherein the induction step is performed for about 163 h.

27-38. (Cancelled)

39. (Previously presented) The method of claim 6, wherein the *Pichia pastoris* comprises a mutation in the amino acid sequence of the diphthamide region of EF-2, wherein the mutation prevents ADP ribosylation of EF-2.

40. (Previously presented) The method of claim 39, wherein the mutation is a

substitution from Glycine to Arginine at position 701 of the amino acid sequence encoded by SEQ ID NO: 13.

41. (New) The method of claim 1, wherein the induction temperature is ramped down to during the first four hours of methanol induction.

42. (New) The method of claim 25, wherein the induction temperature is ramped down to during the first four hours of methanol induction.

43. (New) The method of claim 26, wherein the induction temperature is ramped down to during the first four hours of methanol induction.

44. (New) The method of claim 1, wherein the induction step is carried out at 17.5°C and below for at least 44 hours.

45. (New) The method of claim 44, wherein the induction step is carried out at 17.5°C and below for at least 67 hours.

46. (New) The method of claim 25, wherein the induction step is carried out at 16.5°C and below for at least 44 hours.

47. (New) The method of claim 46, wherein the induction step is carried out at 16.5°C and below for at least 67 hours.

48. (New) The method of claim 26, wherein the induction step is carried out at 15°C and below for at least 44 hours.

49. (New) The method of claim 48, wherein the induction step is carried out at 15°C and below for at least 67 hours.